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A NEW AND EFFICIENT METHOD OF CULTIVATING BACILLUS LEPRÆ FROM THE TISSUES

WITH OBSERVATIONS ON THE DIFFERENT STRAINS OF ACID-FAST BACILLI FOUND IN LEPROUS LESIONS *

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In a previous communication[1] one of us (Duval) stated that the initial multiplication *in vitro* of the bacillus found in leprosy lesions is attended with considerable difficulty unless bits of the infected tissue are transferred to the culture-medium and special methods employed for the breaking down of the tissue protein. In other words, the removed tissue particles must be hydrolyzed before the contained acid-fast bacilli will multiply.

The difficulty in continuing the multiplication in subcultures occurs as soon as the digested tissue material has been exhausted. Therefore it is absolutely essential to substitute at some period in the process a medium which contains split products of protein digestion, such as the amino-acids, until the culture has accustomed itself to more or less saprophytic conditions.

Early in the work on the cultivation of acid-fast bacilli from leprous lesions it was noted that, in the removed tissue bits which had become contaminated with putrefactive bacteria, the contained Hansen bacillus multipled in great profusion whenever the extraneous germs digested or softened the tissue. Under these conditions multiplication occurred regardless of the character of the medium employed and within a wide range of temperature.

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1. Duval: Jour. Exper. Med., 1911, xiii, 365.

This observation led to a study of the rôle played by such extraneous bacteria which established the fact that the multiplication *in vitro* of the acid-fasts was the result of a hydrolytic action on the protein with the attendant appearance of dissociate products. These products of tryptic digestion supplied the necessary nutrient for the lepra bacillus.

Undoubtedly this digestive action on tissue by extraneous bacteria explains the success attained by Clegg[2] in cultivating an acid-fast organism from leprous material which he seeded with amebas and their symbions, the bacteria and not the amebas playing the important rôle. Consequently the deliberate addition of various amino-acids to culture-media was resorted to.

Many of the necessary amino-acids, however, are often difficult to obtain and to prepare into suitable mediums. In view of this fact various nutrients have been experimented with in the hope of finding an efficient medium which would be practicable for this purpose and readily accessible to all laboratories.

Whereas the "hydrolizing method" is thoroughly efficient in establishing the initial growth and is possibly to be preferred where the specific organism is very scarce in the lesions, yet it entails the subsequent use of a special medium in order to obtain pure the Hansen culture. Therefore a medium which will recover the culture directly is certainly to be preferred under ordinary circumstances. Trypsinized egg-medium which was formerly employed for this purpose is of value, but not as certain in all cases as some of the other methods.

In the summer of 1911 one of us (Wellman) suggested the use of mammalian placental tissue extract as a nutrient that might be suitable for the purpose of cultivating *B. leprœ* and allied species which apparently require the dissociate products of tryptic digestion, since the tissue named has been found to be rich in the amino-acids.[3]

We therefore set about the preparation of a medium containing the untreated juice from human placenta in varying proportions and combinations and tested its value in the isolation and cultivation not only of *B. leprœ*, but of various highly specialized parasitic micro-organisms. So far the fluid extract of placenta alone or

2. Clegg: Philippine Jour. Sc., 1909, iv, 403.
3. Wellman: Jour. Biol. Chem., July, 1911, ix, 471; Zentralbl. f. Physiol., xxv, No. 6, p. 267; No. 13, p. 514; No. 20, p. 963.

combined with glycerin-agar has served, in our hands, as an admirable culture-medium for the isolation of *B. leprœ, B. tuberculosis* and the acid-fast organism of rat leprosy.

The medium is prepared as follows: The fresh human placenta is thoroughly macerated and ground up in a meat-chopper, after the blood is washed out by sterile saline run through the attached vessels. To every pound of placental tissue there is added 0.5 liter of sterile salt solution. This mixture is now allowed to infuse for forty-eight hours at refrigerator temperature, when it is filtered through a previously tested No. N Berkefeld filter for purposes of sterilization. To facilitate the filtration the cylinder of the Berkefeld filter is filled with clean, fine sterile sand until the candle is completely submerged. The clear amber-colored filtrate is then tubed or added to a 2 per cent. sterile agar which has been previously melted and cooled to about 41 C. The placental juice and the agar are then thoroughly mixed and the mixture sloped. The addition of 3 per cent. glycerin possibly enhances its nutritive value.

With placental extract either in the form of a liquid medium or of glycerinated agar the acid-fast bacilli in the removed and macerated tissue bits from human or rat leprosy multiply to such an extent that in from five to seven days the growth is distinctly visible to the naked eye.

Not only the initial culture, but subsequent transplants, in the case of *B. leprœ,* flourish on this medium. The growth is profuse and moist and without pigment in some cultures, while others are distinctly chromogenic. The chromogens subsequently will grow on the ordinary laboratory media while the non-pigment-producers refuse to multiply on anything but enriched special media.

It is not the purpose of this paper to discuss or attempt to prove which of these types of cultures are or are not the causal agent in leprosy. Suffice it to say that the acid-fast bacilli seen in the human and rat leprous lesion can in all cases be cultivated where the special method above outlined is employed.

It is, however, of interest in this connection to point out what may be designated as a period of absolute parasitism which seems to persist for some strains of acid-fast cultures recovered from leprous lesions. We

have cultures which do not, thus far, exercise saprophytic growth function, although many generations removed from the parent stem. On the other hand, there are among our cultures strains which at first grow slowly and only on special media, but which become more or less saprophytic after repeated subculturing over a period of months.

This decided difference in biologic features inevitably brings up the questions of their relationship to one another and of their etiologic significance. Do we encounter in the leprous lesion two or more distinct species or does the apparent difference merely represent separate stages in the life-cycle of an extremely pleomorphic organism? Again, it may be asked, are not the subsequent rapid growers extraneous saprophytes while the cultures which conform more to our ideas of a pathogenic species (by growing *in vitro* only under special conditions) represent the real etiologic factor in no way related to the first-mentioned form?

On account of the unreliability of serologic tests for the group of bacteria possessing a fatty or lipoid body (which holds for all the known species in this group) and to the difficulty in producing experimental lesions with the particular organisms under discussion, this problem is rendered difficult of solution.

In the hope of throwing further light on this important question we have undertaken a careful biologic study of representatives of the two types of leprosy organisms above mentioned. The results of this study will form the subject of a subsequent communication.

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